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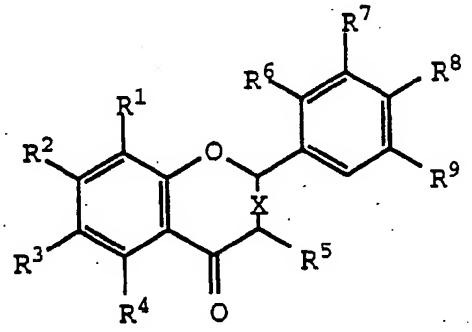
(54) Chondroprotective agents.

(57) A chondroprotective agent comprising a flavonoid compound of the general formula (I):



(I)

wherein R^1 to R^9 are, independently, a hydrogen atom, hydroxyl group, or methoxyl group and X is a single bond or a double bond, or a stereoisomer thereof, or a naturally occurring glycoside thereof is disclosed. The above compound strongly inhibits proteoglycan depletion from the chondrocyte matrix and exhibits a function to protect cartilage, and thus, is extremely effective for the treatment of arthropathy.

EP 0 633 022 A2

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an agent for protecting cartilage, i.e., a chondroprotective agent, more particularly, a chondroprotective agent containing a flavonoid compound or a stereoisomer thereof, or a naturally occurring glycoside thereof.

2. Description of the Related Art

There are various types of arthropathy, for example, rheumatoid arthritis, rheumatic fever, and osteoarthritis. Many people particularly suffer from rhematoid arthritis and osteoarthritis. These diseases have been studied as the major types of arthropathy. There are congenital and secondary osteoarthritis, and further primary osteoarthritis caused by degeneration of the articular cartilage along with aging. Patients suffering from primary osteoarthritis have recently been increasing along with the increase in the population of the aged.

Although there are considerable differences of the causes and conditions between rheumatoid arthritis and osteoarthritis, the articular function becomes eventually obstructed by the destruction of the cartilage in both of rheumatoid arthritis and osteoarthritis.

The first choice of medicines for the treatment of rheumatic diseases such as rheumatoid arthritis, rheumatic fever, systemic lupus erythematosus, and osteoarthritis are analgesic and anti-inflammatory agents, for example, aspirin or indometacin. Further, gold compounds such as Shiosol, immunomodulators, steroids, or D-penicillamine are used as medicines for the treatment of rheumatoid arthritis.

The above conventional analgesic and anti-inflammatory agents, however, were not effective against the destruction of the articular cartilage, and in fact, sometimes exhibited adverse effect in experiments using chondrocytes. Further, no inhibitory effect on articular cartilage destruction was also observed in the above-mentioned medicines for the treatment of rheumatoid arthritis.

It is known that flavonoids may be used as an agent for protecting a blood vessel and further in the following pharmaceutical applications: a virus genome deactivating agent for apigenin, chrysin, morin, fisetin, and baicalein [Japanese Unexamined Patent Publication (Kokai) No. 2-101013], an agent for determining the function of polymorphonuclear leukocyte for flavonoids [Japanese Unexamined Patent Publication (Kokai) No. 63-253254], an oral agent for suppressing smoking for flavonoids [Japanese Unexamined Patent Publication (Kokai) No. 4-46119], treatment of high protein edema for rutin, diosmin, and the like (U.S. Patent No. 5,096,887), an anti-tumor agent containing flavonoids [Japanese Unexamined Patent Publication (Kokai) No. 3-275625], an anti-tumor agent containing apigenin [Japanese Examined Patent Publication (Kokoku) No. 3-61644], an agent for suppressing the formation of peroxylipid for hesperetin, kaempferol, and the like [Japanese Unexamined Patent Publication (Kokai) No. 3-5423], an anti-tumor agent containing kaempferol [Japanese Unexamined Patent Publications (Kokai) No. 4-103529 and No. 4-103532], a calcium antagonist for hesperidin and luteolin [Japanese Unexamined Patent Publication (Kokai) No. 4-243822], a sialidase inhibitor for luteolin [Japanese Unexamined Patent Publication (Kokai) No. 64-42427], an anti-retrovirus agent for luteolin [Japanese Unexamined Patent Publication (Kokai) No. 3-7224], an anti-HBV (hepatitus B virus) agent for quercetin [Japanese Unexamined Patent Publication (Kokai) No. 4-234320], and the like.

Flavonoids have not, however, been known to be useful as chondroprotective agents.

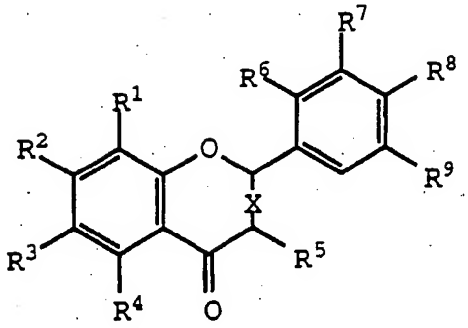
SUMMARY OF THE INVENTION

The present inventors engaged in intensive research to develop a chondroprotective agent for suppressing the destruction of the articular cartilage and as a result found that the particular flavonoid compounds and stereoisomers thereof, and the naturally occurring known glycosides thereof showed significant inhibition of the depletion of proteoglycan which is a major component of the cartilage matrix, and therefore, are useful as a chondroprotective agent for prohibiting the destruction of the articular cartilage.

Accordingly, the object of the present invention is to provide a chondroprotective agent containing as an active ingredient a particular flavonoid compound or a stereoisomer thereof, or a naturally occurring known glycoside thereof.

Other objects and effects of the present invention will be clear from the following description.

The present invention relates to a chondroprotective agent comprising a flavonoid compound of the general formula (I):



(I)

wherein R^1 to R^9 are, independently, a hydrogen atom, hydroxyl group, or methoxyl group and X is a single bond or a double bond, or a stereoisomer thereof, or a naturally occurring glycoside thereof (hereinafter referred to as "the present substance").

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The active ingredient of the chondroprotective agent according to the present invention is a flavonoid, which is widely present in the vegetable kingdom. Typical flavonoid compounds include flavones, flavonols, flavanones, and flavanonols. Flavanones contain an asymmetric carbon atom at the 2-position, and flavanonols contain asymmetric carbon atoms at the 2- and 3-positions, and such compounds may be present as the stereoisomers. These stereoisomers can also be used in the present invention. Further, the saccharides present in the above naturally occurring glycosides are not particularly limited. As examples of the naturally occurring glycosides, there may be mentioned glucoside, galactoside, fructoside, rhamnoside, rutinoside (that is, rhamnoglucoside), arabinoside, xyloside, apioglucoside, and robinobioside.

In the present invention, any naturally occurring flavonoids may be used as the above present substance. The flavonoid compounds and naturally occurring glycosides thereof shown in the following Table 1 are preferable.

Table 1

No.	Name	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	X
1	Flavone	H	H	H	H	H	H	H	H	H	Double
2.	Chrysin	H	OH	H	OH	H	H	H	H	H	ditto
3.	Toringin	H	OH	H	OGlu	H	H	H	H	H	ditto
4.	Primetin	OH	H	H	OH	H	H	H	H	H	ditto
5	Apigenin	H	OH	H	OH	H	H	H	OH	H	ditto
6	Cosmosiin	H	OGlu	H	OH	H	H	H	OH	H	ditto
7	Apiin	H	OApg	H	OH	H	H	H	OH	H	ditto
8	Luteolin	H	OH	H	OH	H	H	OH	OH	H	ditto
9	Galuteolin	H	OH	H	OGlu	H	H	OH	OH	H	ditto
10	Gluco -luteolin	H	OGlu	H	OH	H	H	OH	OH	H	ditto
11	Acacetin	H	OH	H	OH	H	H	H	OCH_3	H	ditto
12	Linarin	H	ORut	H	OH	H	H	H	OCH_3	H	ditto
13	Diosmetin	H	OH	H	OH	H	H	OH	OCH_3	H	ditto
14	Diosmin	H	ORut	H	OH	H	H	OH	OCH_3	H	ditto
15	Baicalein	H	OH	OH	OH	H	H	H	H	H	ditto
16	Fisetin	H	OH	H	H	OH	H	H	OH	H	ditto
17	Kaempferol	H	OH	H	OH	OH	H	H	OH	H	ditto
18	Trifolin	H	OH	H	OH	OGal	H	H	OH	H	ditto
19	Astragalin	H	OH	H	OH	OGlu	H	H	OH	H	ditto

Table 1 (continued)

No.	Name	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	X
20	Robinin	H	ORha	H	OH	ORob	H	H	OH	H	Double
21	Kaem -pferitrin	H	ORha	H	OH	ORha	H	H	OH	H	ditto
22	Quercetin	H	OH	H	OH	OH	H	OH	OH	H	ditto
23	Quercitrin	H	OH	H	OH	ORha	H	OH	OH	H	ditto
24	Iso -quercitrin	H	OH	H	OH	OGlu	H	OH	OH	H	ditto
25	Rutin	H	OH	H	OH	ORut	H	OH	OH	H	ditto
26	Morin	H	OH	H	OH	OH	OH	H	OH	H	ditto
27	Myricetin	H	OH	H	OH	OH	H	OH	OH	OH	ditto
28	Myricitrin	H	OH	H	OH	ORha	H	OH	OH	OH	ditto
29	Datiscetin	H	OH	H	OH	OH	OH	H	H	H	ditto
30	Quer -cetagetin	H	OH	OH	OH	OH	H	OH	OH	H	ditto
31	Quer -cetagitrin	H	OGlu	OH	OH	OH	H	OH	OH	H	ditto
32	Rhamnetin	H	OCH_3	H	OH	OH	H	OH	OH	H	ditto
33	Iso -rhamnetin	H	OH	H	OH	OH	H	OCH_3	OH	H	ditto
34	Pinocembrin	H	OH	H	OH	H	H	H	H	H	Single
35	Naringenin	H	OH	H	OH	H	H	H	OH	H	ditto
36	Salipurpin	H	OH	H	OGlu	H	H	H	OH	H	ditto
37	Prunin	H	OGlu	H	OH	H	H	H	OH	H	ditto
38	Naringin	H	ORha	H	OH	H	H	H	OH	H	ditto
39	Sakuranetin	H	OCH_3	H	OH	H	H	H	OH	H	ditto
40	Sakuranin	H	OCH_3	H	OGlu	H	H	H	OH	H	ditto
41	Hesperetin	H	OH	H	OH	H	H	OH	OCH_3	H	ditto
42	Hesperidin	H	ORut	H	OH	H	H	OH	OCH_3	H	ditto
43	Eriodictyol	H	OH	H	OH	H	H	OH	OH	H	ditto
44	Eriodictin	H	ORha	H	OH	H	H	OH	OH	H	ditto
45	Pinobanksin	H	OH	H	OH	OH	H	H	H	H	ditto
46	Arom -adendrin	H	OH	H	OH	OH	H	H	OH	H	ditto
47	Engelitin	H	OH	H	OH	ORha	H	H	OH	H	ditto
48	Fustin	H	OH	H	H	OH	H	OH	OH	H	ditto
49	Taxifolin	H	OH	H	OH	OH	H	OH	OH	H	ditto

Table 1 (continued)

No.	Name	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	X
50	Astilbin	H	OH	H	OH	ORha	H	OH	OH	H	Single
51	Ampelopsin	H	OH	H	OH	OH	H	OH	OH	OH	ditto

OGlu: Glucoside, OApg: Apioglucoside, ORut: Rutinoside, OGal: Galactoside, ORha: Rhamnoside, ORob: Robinobioside

The compounds Nos. 34 to 51 in Table 1 include a single bond as X. Thus, the carbon atom in the 2-position or the carbon atoms in the 2- and 3-positions are asymmetrical, and there exist stereoisomers. It is known that pinocembrin includes (±) and (S) isomers; naringenin, sakuranetin, hesperetin and eriodictyol include (±), (R), and (S) isomers; pinobanksin includes (2R-trans) and (2S-trans) isomers; aromadendrin and fustin include trans-(±), (2R-trans), and (2S-trans) isomers; taxifolin and ampelopsin include trans-(±), (2R-trans), (2S-trans), and (2R-cis) isomers.

It is possible to use, as the flavonoids, compounds isolated and purified from naturally occurring plants or chemically synthesized. Many compounds described in Table 1 are commercially available. For example, it is possible to obtain flavone, apigenin, luteolin, acacetin, linarin, diosmetin, baicalein, fisetin, kaempferol, quercetin, hesperetin, and hesperidin from Funakoshi Co., Ltd., Tokyo.

Examples of the acute toxicity of the present substance are as follows: Mouse LD_{50} of quercetin (oral administration): 160 mg/kg and mouse LD_{50} of fisetin (intravenous injection): 180 mg/kg.

Further, no abnormalities were observed for a week after hesperetin was administered orally to BALB/c mice (female, seven weeks old) at the dose of 100 mg/kg. The same results were obtained where hesperidin, acacetin, diosmetin, apigenin, luteolin, or kaempferol was administered.

As a pharmacological effect, the present substance exhibits the function to inhibit destruction of chondrocyte matrix in chondrocyte culture (derived from cartilage of rabbit shoulder and knee joints) (see Example 1 as below).

Accordingly, the present substance is useful as a chondroprotective agent for treating various types of arthropathy accompanying the cartilage destruction of joints. Examples of such arthropathy are rheumatoid arthritis, osteoarthritis, periarthritis humeroscapularis, shoulder-arm-neck syndrome, lumbago, etc.

The chondroprotective agent containing the present substance as an active ingredient may be in the form of any conventional formulation. The chondroprotective agent may contain the present substance alone, or a mixture of the present substance with any pharmaceutically acceptable carrier or diluent. The chondroprotective agent may contain the active ingredient in an amount of 0.01 to 100 percent by weight, preferably 0.1 to 70 percent by weight.

The chondroprotective agent of the present invention may be administered orally or by some other routes.

The dose of the chondroprotective agent according to the present invention varies with the patient (animal or human), age, individual differences, state of illness, and the like. Generally speaking, however, when a human is treated, the dose of oral administration of the present substance is in the range of 0.1 to 500 mg/kg (body weight) per day, preferably 0.5 to 200 mg/kg (body weight), which is usually divided into 1 to 4 dosages in a day, although the dose outside the above range may sometimes be administered.

EXAMPLE

The present invention now will be further illustrated by, but is by no means limited to, the following Examples.

Example 1: Effect of Test Compounds on Proteoglycan Depletion in Chondrocyte Culture

(a) Preparation of Cultured Chondrocytes

The cartilages were sterilely extracted from the shoulder and knee joints of rabbits (New Zealand White Rabbits) (body weight of 1 to 1.5 kg). The cartilages were thoroughly washed with PBS (-) (free of Ca^{2+}, Mg^{2+}), Hanks' solution and 0.1% EDTA-PBS (-), and then cut into small segments (1 mm x 1 mm x 1mm). After PBS (-) containing 0.1% EDTA was added, the segments were allowed to stand in an incubator of 37 • C for 30 minutes. Then, the segments were treated with a trypsin solution (0.25%) at 37 • C for one hour to remove the connective tissue adhered to the cartilage. After the supernatant had been removed, the cartilages were treated for about 2 to 2.5 hours in a Ham F-12 medium containing 10 % fetal bovine serum (FBS) and 0.2 % collagenase. Then, the collagenase solution was centrifuged (1500 r.p.m.), and the residual chondrocytes were washed twice with a Ham F-12 medium (chondrocyte culture medium) containing 10 % FBS. Finally, the resulting suspension was adjusted so that the chondrocytes were suspended in the concentration of 3×10^5 cells/ml in the chondrocyte culture medium. The chondrocytes were seeded in an amount of 1 ml/well on 24-well plates. The chondrocytes became confluent after 4 days. The experiment were performed within two weeks after reaching the confluent stage.

(b) Addition of Compounds to be tested and Proteoglycan Depleting Agents

The chondrocyte culture medium which had been used for cultivating the chondrocytes was removed from each well and 800 µl of fresh serum-free S-Clone medium containing 0.1% human serum albumin was added. Further, 100 µl of S-Clone medium containing the compounds to be tested (containing the compound in the concentration of 10 fold the final concentration; DMSO concentration = 2.5%) was added. The chondrocytes were cultured in the presence of carbon dioxide (5%) and air (95%) for 2 hours. Then, the proteoglycan depleting agent, PMA (phorbol myristate acetate) (final concentration = 0.1 µg/ml) was added into the culture medium of the chondrocytes.

The compounds to be tested were as follows:

Compounds of present invention: apigenin (present substance No. 5), luteolin (present substance No. 8), acacetin (present substance No. 11), linarin (present substance No. 12), diosmetin (present substance No. 13), baicalein (present substance No. 15), fisetin (present substance No. 16), kaempferol (present substance No. 17), quercetin (present substance No. 22), hesperetin (present substance No. 41, (S) isomer), and hesperidin (present substance No. 42, (S) isomer) (all from Funakoshi Co.)

Comparative substance: Indometacin (Sigma Chemical Co.)

(c) Determination of proteoglycan

Proteoglycan depletion was determined by the measurement of the glycosaminoglycan (major constituent of proteoglycan, hereinafter referred to as GAG) content following digestion of the chondrocyte matrix with papain.

After 2 days, the supernatant of the chondrocyte culture was removed. Then, 1 ml of 0.03& papaine solution was added to the remaining chondrocyte matrix layer and a reaction was performed at 65 • C for 1 hour to liberate the GAG from the matrix layer. The content of the GAG in the treated papaine solution was determined by the 1,9-dimethylmethylene blue method (refer to R.W. Farndale, Biochim. Biophys. Acta., Vol. 883, pp. 173 to 177, 1986). The GAG content in the chondrocyte matrix of the control test wherein the proteoglycan depleting agent was not added was shown as "100", and the relative amount of the GAG of each experiment except the control test was calculated by by following formula:

$$\text{GAG relative amount (\%)} = (B/A) \times 100$$

wherein A represents the GAG content of the control tests wherein the proteoglycan depleting agent was not added, and B represents the GAG content wherein the proteoglycan depleting agents were added alone or the GAG content wherein the proteoglycan depleting agents and the compounds to be tested were added.

The GAG contents of the control tests varied in a range of 11.23 to 59.0 µg/ml, depending on the period from the time when the chondrocytes became confluent until the time when the chondrocytes were used in the above experiment.

The results are shown in Table 2. The GAG content is the value of the mean value ± standard error (n = 3 to 6). For each of the compounds to be tested, the control test and the proteoglycan depleting test wherein the proteoglycan depleting agent was added were carried out and the results thereof are also shown. The significance was determined by Student's t-test with respect to the proteoglycan depleting test wherein the proteoglycan depleting agent was added. The results of the determination are shown as follows:

*: $P < 0.05$;

**: $P < 0.01$;

***: $P < 0.001$.

In comparison with the GAG content in the control tests wherein the proteoglycan depleting agent was not added, the addition of the proteoglycan depleting agents, PMA, induced a loss of GAG content. Under these conditions, the present compound significantly inhibited or reduced the loss of GAG content, and showed a function to inhibit or suppress the proteoglycan depletion. On the other hand, indomethacin, a conventional analgesic and anti-inflammatory agent, did not show the function to inhibit or suppress the proteoglycan depletion, but caused a significant exacerbation on the proteoglycan depletion.

Table 2

Samples	GAG content (μg/ml)	(Relative amount of GAG) (%)
Control	54.7±0.8***	(100)
PMA	16.5±0.7	(30.2)
PMA + No. 5 (100 μM)	33.3±0.7***	(60.9)
Control	54.8±0.5***	(100)
PMA	15.2±0.6	(27.7)
PMA + No. 8 (100 μM)	28.6±0.5***	(52.2)
PMA + No. 17 (100 μM)	30.5±0.3***	(55.7)
Control	54.0±1.2***	(100)
PMA	20.0±0.4	(37.0)
PMA + No. 11 (100 μM)	24.2±0.3	(44.8)
PMA + No. 16 (100 μM)	30.1±0.9***	(55.7)
Control	55.3±0.6***	(100)
PMA	17.5±0.7	(31.6)
PMA + No. 12 (100 μM)	19.8±0.7*	(35.8)
PMA + No. 13 (100 μM)	27.1±0.7***	(49.0)
Control	11.23±0.2***	(100)
PMA	4.94±0.1	(44.0)
PMA + No. 15 (100 μM)	7.67±0.5*	(68.3)
Control	56.1±0.8***	(100)
PMA	20.4±0.7	(36.4)
PMA + No. 22 (100 μM)	31.0±0.6***	(55.3)
PMA + No. 42 (100 μM)	24.0±0.6**	(42.8)
Control	59.0±0.9***	(100)
PMA	21.1±0.6	(35.8)
PMA + No. 41 (100 μM)	28.7±0.4***	(48.6)
Control	28.0±0.7***	(100)
PMA	15.4±0.5	(55.0)
PMA + indometacin		
(10 μM)	13.2±0.6*	(47.1)
(33 μM)	11.7±0.8**	(41.8)

Example 2: Formulation of Granule

The following ingredients were mixed homogeneously:

Apigenin	20 parts by weight
Lactose	68 parts by weight
Low-substituted hydroxypropylcellulose	10 parts by weight
Hydroxypropylcellulose	2 parts by weight

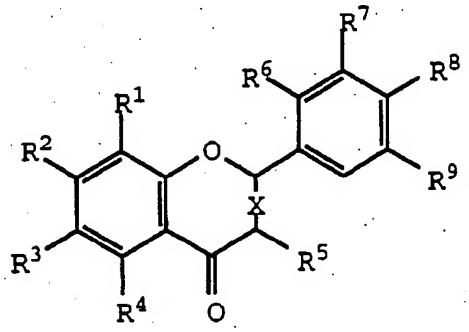
The mixture was kneaded using 32 parts by weight of a wetting agent, ethanol. Then, the kneaded mixture was glanulated by wet granulation and dried to obtain the granule.

As explained above, the present substance strongly inhibits proteoglycan depletion from the chondrocyte matrix and exhibits a function to protect cartilage. Further, the present substance has low toxicity. Accordingly, the present substance is very useful for the treatment of arthropathy, such as rheumatoid arthritis, osteoarthritis, periarthritis humeroscapularis, shoulder-arm-neck syndrome, lumbago, and so on.

Although the present invention has been described with reference to specific embodiments, various changes and modifications obvious to those skilled in the art are deemed to be within the spirit, scope, and concept of the invention.

Claims

1. A chondroprotective agent comprising a flavonoid compound of the general formula (I):



(I)

wherein R^1 to R^9 are, independently, a hydrogen atom, hydroxyl group, or methoxyl group and X is a single bond or a double bond, or a stereoisomer thereof, or a naturally occurring glycoside thereof, and a pharmaceutically acceptable carrier.

2. A chondroprotective agent according to claim 1, wherein X is a double bond.

3. A chondroprotective agent according to claim 2, wherein said flavonoid compound is one or more compounds selected from the group consisting of flavone, chrysin, toringin, primetin, apigenin, cosmosiin, apiin, luteolin, galuteolin, glucoluteolin, acacetin, linarin, diosmetin, diosmin, baicalein, fisetin, kaempferol, trifolin, astragalin, robinin, kaempferitrin, quercetin, quercitrin, isoquercitrin, rutin, morin, myricetin, myricitrin, datiscetin, quercetagetin, quercetagitrin, rhamnetin, and isorhamnetin.

4. A chondroprotective agent according to claim 1, wherein X is a single bond.

5. A chondroprotective agent according to claim 4, wherein said flavonoid compound is one or more compounds selected from the group consisting of pinocembrin, naringenin, salipurpin, prunin, naringin, sakuranetin, sakuranin, hesperetin, hesperidin, eriodictyol, eriodictin, pinobanksin, aromadendrin, engelitin, fustin, taxifolin, astilbin, and ampelopsin.

6. A chondroprotective agent according to claim 1, wherein said naturally occurring glycoside is one or more compounds selected from the group consisting of glucoside, galactoside, fructoside, rhamnoside, rutinoside, arabinoside, xyloside, apioglucoside, and robinobioside.

7. A chondroprotective agent according to claim 1, wherein said flavonoid compound is one or more compounds selected from the group consisting of apigenin, luteolin, acacetin, linarin, diosmetin, baicalein, fisetin, keampferol, quercetin, hesperetin (S-form), and hesperidin (S-form).

8. A chondroprotective agent according to claim 1, wherein said flavonoid compound, stereoisomer thereof, or naturally occurring glycoside thereof is an extract from a naturally occurring material.

9. Use of a compound of the general formula (I), or a stereoisomer thereof, or a naturally occurring glycoside thereof according to claim 1, for preparing a chondroprotective agent.

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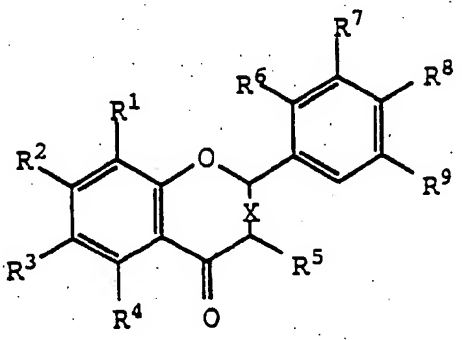
(54) Chondroprotective agents.

(57) A chondroprotective agent comprising a flavonoid compound of the general formula (I):



(I)

wherein R^1 to R^9 are, independently, a hydrogen atom, hydroxyl group, or methoxyl group and X is a single bond or a double bond, or a stereoisomer thereof, or a naturally occurring glycoside thereof is disclosed. The above compound strongly inhibits proteoglycan depletion from the chondrocyte matrix and exhibits a function to protect cartilage, and thus, is extremely effective for the treatment of arthropathy.

EP 0 633 022 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 10 9872

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	AGENTS AND ACTIONS, vol. 12, page 298 O.P. AGARWAL 'The anti-inflammatory action of nepitrin, a flavonoid' * See the whole document, especially Table 2 and penultimate paragraph of the discussion *	1,2,9	A61K31/365 A61K31/70
Y	* " *	1-3,6-9	

Y	Z. RHEUMATOL., vol. 42, page 203 G. WILHELMI ET AL. 'zur Prüfung potentieller Antiarthrotika an der spontanen Arthrose der Maus' *see the whole document *	1-3,6-9	

X	CHEM. PHARM. BULL., vol. 32, page 2724 M. KUBO ET AL. 'Studies on scutellariae radix. VII Anti-arthritic and anti-inflammatory actions of methanolic extract and flavonoid components from scutellariae radix' * see the whole document, especially Table 4 *	1-3,6-8	
Y	* " *	9	

Y	US-A-4 268 517 (NIEBES ET AL.) 19 May 1981 * see the whole document*	1-3,6-9	

	-/--		

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	15 May 1995	Isert, B

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 10 9872

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	ARCH. PHARM. RES., vol. 16,no. 1, 1993 pages 25-28, S.J. LEE ET AL. 'antiinflammatory activity of naturally occurring flavone and flavonol glycosides' * see the whole document, especially Figure 1 and Table 1 *	1-4,6-8	
Y	* " "	9	

X	FITOTERAPIA , vol. 90,no. 5, 1990 pages 460-461, G.B.S. REDDY ET AL. 'comparison of the anti-inflammatory activity of certain methoxyflavonoids' * see the summary *	1-3,6-8	
Y	* " *	9	
	---		TECHNICAL FIELDS SEARCHED (Int.Cl.6)
X,P	INDIAN J. PHARMACOL. SCI., vol. 55,no. 5, September 1993 pages 180-183, N.S. MUTHIAH ET AL. 'Anti-inflammatory activity of flavone and its methoxy derivatives- a structure activity study-' * see the summary and Table 2 *	1-4,6-8	
X,P	* " *	9	

A	EP-A-0 395 441 (KUREHA KAGAKU KOGYO) 31 October 1990 * see page 8, line 36 - page 9, line 30; claims 1-8,10 *	1-9	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
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EPO FORM 1503 03.82 (P04C01)

European Patent Office

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

See sheet -B-

☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid,

namely claims:

☐ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

namely claims:

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims 1 (partially), 2,3,6-9 (partially):
   Chondroprotective flavones

2. Claims 1 (partially), 4,5,6-9 (partially):
   Chondroprotective flavanones